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Cardiac dysfunction in rats prone to audiogenic epileptic seizures

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ABSTRACT

Purpose: Cardiac dysfunction is one of the possible causes of sudden unexpected death in epilepsy (SUDEP). Therefore, the objective of this study was to evaluate cardiac and electrocardiographic parameters in rats with audiogenic epileptic seizures (WAR – Wistar audiogenic rats).
Methods: *In vivo* arterial pressure, heart rate (HR), autonomic tone and electrocardiography (ECG) were measured in awake animals in order to examine cardiac function and rhythm. *Ex vivo*, the Langendorff technique was used to analyze the cardiac function and the severity of reperfusion arrhythmias. *In vitro*, confocal microscopy was used to evaluate calcium transient parameters of isolated ventricular cardiomyocytes.
Results: *In vivo* autonomic tone evaluation revealed enhanced sympathetic activity, changes in cardiac function with increased systolic arterial pressure and higher basal HR in WAR. In addition, ECG analysis demonstrated electrical alterations with prolongation of the QT interval and QRS complex in these animals. *Ex vivo*, we observed a decrease in systolic tone and HR and an increase in the duration of ischemia/reperfusion arrhythmias in WAR. Moreover, intracellular Ca^{2+} handling analysis revealed an increase in the peak of calcium and calcium transient decay in audiogenic rats. Treatment with atenolol (β1-adrenergic antagonist) normalized the systolic tone, reduced cardiac hypertrophy and the associated increase in the susceptibility to reperfusion arrhythmias observed in WAR.
Conclusion: We present evidence that chronic disturbances in sympathetic tone in WAR cause increases the risk to life-threatening arrhythmias. Our results support a relationship between seizures, cardiac dysfunction and cardiac arrhythmias, which may contribute to the occurrence of SUDEP.

1. Introduction

The mortality among patients with epilepsy is on average 2–3 times greater than that in the general population.[1] The commonest cause of death in young adults with epilepsy is sudden unexpected death in epilepsy (SUDEP). The underlying mechanisms that cause SUDEP are still unclear. Several different mechanisms may be involved, and there may be no single explanation for all cases. For example, one mechanism, which has been suggested, involves cerebrogenic cardiac arrhythmia and autonomic dysfunction.[2] There are limited observations of SUDEP with case reports of observed cardiac arrhythmias.[3] It has been suggested that SUDEP may be caused by dysfunction of the cardiovascular autonomic system, which exposes the patient to arrhythmias and sinus arrest.[4]

The development of cardiovascular autonomic dysfunction during the interictal period has been shown to be associated with the epileptogenic activity.[1] Experimental data suggest that interictal epileptogenic activity induces autonomic imbalance, which may be associated with cardiac arrhythmias.[5] Autonomic symptoms frequently occur during epileptic seizures either as an accompaniment to other seizure symptoms or as the predominant seizure manifestation.[6]

Cardiac rhythm and conduction abnormalities are common during seizures, particularly if the seizure is prolonged or generalized.[7] Electrocardiogram (ECG) abnormalities with potentially serious changes including ST-depression and T-wave inversion were observed in patients during the ictal and postictal period, suggesting that myocardial ischemia associated with ictal sympathetic storms may lead to lethal arrhythmias.[7,8] Natelson et al.[9] observed that patients with epilepsy, who died suddenly and

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unexpectedly presented cardiac pathological conditions that could be responsible for their deaths. In addition, Tigaran et al.[10] suggested secondary cardiac damages in epileptic patients since they found signs of ischemia on ECG and elevated cardiac enzymes (troponin). Alehan et al.[11] showed presence of elevated brain natriuretic peptide (BNP) and creatine kinase (CK-MB) in patients with seizures, the first evidence of subtle cardiac dysfunction in epilepsy patients.

In a previous study,[12] we observed that convulsive seizures triggered by maximal electroshock (MES) induced profound abnormalities in cardiac rhythm and increased the incidence/duration of ischemia-reperfusion arrhythmias in Wistar rats. Furthermore, Metcalf et al.[13] suggested that status epilepticus produces tachycardic ischemia, following the activation of the sympathetic nervous system in rats, resulting in cardiac myofilament damage, arrhythmogenic alterations in cardiac electrical activity, and increased susceptibility to ventricular arrhythmias. Fazan Jr et al.[14] observed that autonomic imbalance (sympathetic predominance) in Wistar audiogenic rats (WAR) might be associated with an increased risk of life-threatening cardiovascular events in this strain. Therefore, taking into consideration the disruption of the normal autonomic control and cardiac alterations suggestive of myocardial injury during epileptic seizures, the aim of this study was to evaluate the cardiovascular and electrocardiographic parameters of rats predisposed to seizures induced by audiogenic stimulus.

2. Methods

2.1. Animals

Experiments were performed in male Wistar rats (250–300 g, $n = 22$) from the main breeding stock of the Institute of Biological Sciences (Federal University of Minas Gerais, Brazil) and WAR (250–300 g, $n = 46$) from the inbred colony maintained at the Department of Physiology and Biophysics (Institute of Biological Sciences, Federal University of Minas Gerais, Brazil). The WAR strain is a genetic model of sound-induced reflex epilepsy that, in the acute situation, mimics tonic-clonic seizures (audiogenic seizures). All animals were housed individually in plastic cages, under controlled lighting conditions (lights on at 6:00 am and off at 8:00 pm), room temperature at 24 °C and with food and water *ad libitum*. All efforts were made to avoid any unnecessary distress to the animals and all animal procedures were performed in accordance with institutional guidelines approved by the Ethics Committee in Animal Experimentation of the Federal University of Minas Gerais, Brazil (CETEA-UFMG), which are in accordance with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals.

2.2. Acoustic stimulation and behavioral evaluation of seizure severity

The apparatus used to induce acoustic stimulation consisted of a cylindrical transparent cage inside a larger, sound-proof box, provided with a door and frontal glass window for observation. A sound stimulus (120 dB SPL) was delivered into the acoustic chamber through a loudspeaker until tonic seizures appeared or during a maximum period of 1 min. Behavior was assessed by direct observation using a set of discrete behavioral categories and quantified by means of a severity index (SI) scale ranging from zero to one.[15] All animals were submitted to a screening procedure (at 70, 74, and 78 days of age) in order to determine seizure severity and to evaluate audiogenic susceptibility. The WAR display epileptic behavior after sound stimulation, presenting running fits, jumping and atonic falling, followed by tonic-clonic seizures and clonic spasms. The tests were always conducted after 4:00 pm and all seizures were induced since these animals do not have spontaneous seizures.[16] A resting period of at least one week after screening was allowed before the initiation of the experimental protocols. The most frequent behavioral sequences produce the following SI values: 0.11 = wild running with only one running fit; 0.23 = wild running with only one running fit, jumping and atonic falling; 0.38 = wild running with two running fits, jumping and atonic falling; 0.61 = all of the above plus tonic convulsion (back arching tonus); 0.73 = all the above plus partial (only forelimb or hindlimb) and generalized (forelimb and hindlimb) clonic seizures; 0.85 = all the above plus clonic spasms; 0.90 = all the above plus ventral flexion of the head; 0.95 = all the above plus forelimb hyperextension; 1.0 = all the above plus forelimb and hindlimb hyperextension.[17] In the present study only animals with SI > 0.85 (running, jumping plus clonic spasms) were included in the experimental groups.

2.3. Arterial pressure measurements

Five Wistar rats and five WAR were used for recording arterial pressure and heart rate (HR). Twenty-four hours before the experiments, the animals were anesthetized with 2.5% tribromoethanol (1 mL/100 g of body weight, i.p., Sigma–Aldrich, Inc.) and polyethylene catheters (PE-10 connected to PE-50) were inserted into abdominal aorta through the left femoral artery and into the femoral vein for recording arterial pressure and drug infusion, respectively. The catheters were tunneled subcutaneously and exteriorized backing the cervical region of the animal. The hemodynamic parameters: pulse arterial pressure (PAP), mean arterial pressure (MAP) and systolic and diastolic arterial pressure (SAP and DAP, respectively) were monitored simultaneously during experiments by a solid-state strain gauge transducer (TSD 104A, Biopac Systems, Inc., CA, USA). The HR was determined from the SAP. The transducer was connected to a computer through a data acquisition system (MP100; Biopac Systems, Inc., CA, USA). The data were analyzed by the AcqKnowledge Software. The experiments were conducted in conscious and freely moving rats.

2.4. Vagal and sympathetic activity evaluation

After the measurement of the arterial pressure and HR, the vagal and sympathetic activities were assessed in the same animals, *i.e.* five Wistar rats and five WAR. Before drug administration, HR and MAP were monitored during 20 min (baseline period) in conscious freely moving rats. After stabilization, vagal and sympathetic activities were assessed by intravenous injections of methylatropine (MA; 3 mg/kg; Sigma–Aldrich, Inc.) or atenolol (4 mg/kg; Sigma–Aldrich, Inc.) at a maximal volume of 0.2 mL per injection. On the first day, MA was injected after recording of the resting HR. Because the HR response to MA reached the peak within 10–15 min, this time interval was standardized for measurement of HR. Atenolol was injected 15 min after MA injection and the responses were measured after 10–15 min. To obtain the reverse sequence of the blockade, atenolol was administered before the application of MA in the second day of experiment. The efficacy of the blockade induced by MA and atenolol was confirmed by the elimination of the reflex changes in HR produced by phenylephrine (8 μg/kg; Sigma–Aldrich, Inc.) and sodium nitroprusside (50 μg/kg; Sigma–Aldrich, Inc.) administration, respectively, at a maximal volume of 0.2 mL per injection. The autonomic tone was evaluated as previously described.[18] A diagram showing the protocol used to determine the cardiac sympathetic and parasympathetic tone and effects is presented in Fig. 1. At the end of the protocol, the wet weight of the hearts was recorded, normalized by the tibia length and then expressed as mass index (mg/mm).

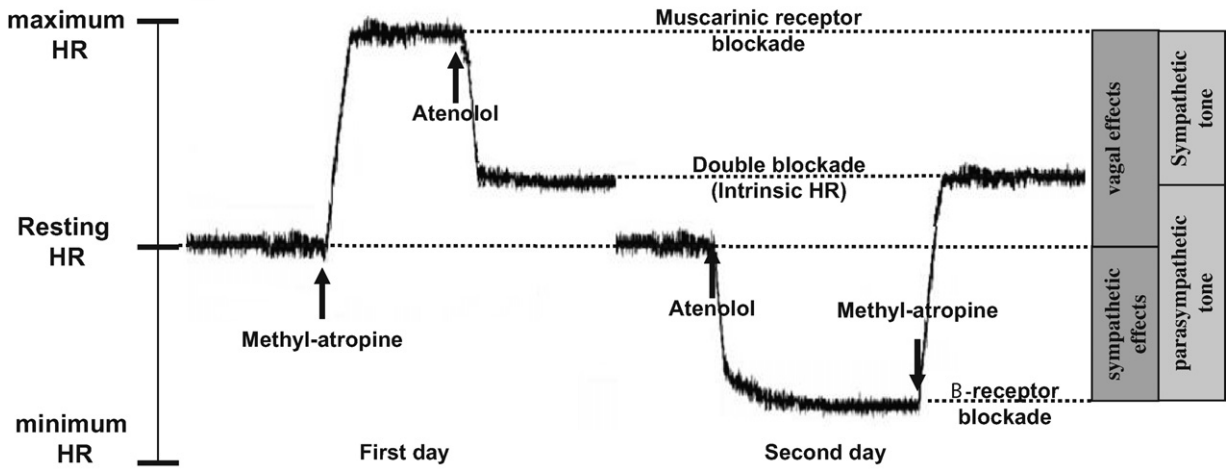

Fig. 1. Diagram showing the protocol used to determine the cardiac sympathetic and parasympathetic tone and effects. Cardiac sympathetic and parasympathetic tone and effects were obtained based on the heart rate (HR) responses to methylatropine [muscarinic cholinergic receptor (M-ChR) blocker (*i.e.*, MA)] and atenolol [β-adrenergic receptor (β-AdR) blocker]. The sympathetic tone was calculated as the difference between the maximum HR after the blockade with MA and the intrinsic HR (IHR) (residual sympathetic tone in the absence of vagal tone). Conversely, the parasympathetic tone was calculated as the difference between the IHR and the minimum HR after the blockade with atenolol (residual vagal tone in the absence of sympathetic tone). The IHR was obtained when the double blockade was performed. Furthermore, the vagal effect was obtained by the difference between maximum HR after MA injection and basal HR and the sympathetic effect was determined as the difference between basal HR and the minimum HR after atenolol injection.

2.5. Electrocardiogram analysis

To obtain ECG tracings, bipolar platinum electrodes were positioned in the thorax (subcutaneous tissue) directly in derivation DII. The ECG recordings were performed 24 h after the implantation of the electrodes and evaluated in unanesthetized, freely moving rats. To determine the intervals RR, PR, QT, corrected QT (QTc) and QRS complex, a period of 10 s were analyzed in the ECG tracing of each animal. The QT interval was measured starting from the onset of the QRS complex until the end of the T wave, which is the return of the T wave to the baseline.[19] QTc was obtained using Bazett's formula ($QTc = QT/\sqrt{RR}$).[20] The vagal sympathetic effect (VSE) was calculated and defined as the ratio between RR interval and intrinsic RR interval (during autonomic blockade), in accordance to Goldberger.[21] Furthermore, a $VSE > 1$ reflects vagal predominance and a $VSE < 1$ reflects sympathetic predominance.

2.6. Isolated hearts preparation and induction of reperfusion arrhythmias

The rats were decapitated 10–15 min after intraperitoneal injection of 400 IU heparin. The thorax was opened, the heart was rapidly excised and immediately cooled in iced buffer and perfused through an aortic stump with Krebs-Ringer solution (KRS) containing: 118.3 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4{\cdot}7H_2O$, 2.5 mM $CaCl_2{\cdot}2H_2O$, 11.7 mM glucose and 26.5 mM $NaHCO_3$.[22] The perfusion fluid was maintained at $37 \pm 1\,^{\circ}C$ with a constant pressure (75 mmHg) and oxygenation (5% CO_2/95% O_2). A force transducer (model FT3, Grass) was attached through a heart clip to the apex of the ventricles to record the contractile force (tension, g) in a computer using a data acquisition system (Biopac System, Inc., CA, USA). Electrical activity was recorded utilizing an ECG with the aid of two platinum electrodes placed directly on the surface of the right atrium and left ventricle (bipolar lead). A diastolic tension of 1.0 ± 0.1 g was applied to the hearts. The coronary flow was measured by collecting the perfusate over a period of 1 min at regular intervals.[23] The hearts were perfused for an initial 30-min period with KRS under sinusal rhythm. At the end of this time, a ligature was placed around the left anterior descending (LAD) coronary artery close to its origin. Both ends of the ligature were passed through a small plastic cylinder, which was then pressed against the artery. The resulting arterial occlusion was maintained for 15 min by clamping the plastic cylinder and ligature. After the 15-min period of coronary occlusion, we reperfused the heart by removing the clamp and the tube. Reperfusion rhythm disturbances were then monitored for 30 min.[24] Cardiac arrhythmias were defined as the presence of ventricular tachycardia (VT) and/or ventricular fibrillation (VF) after the ligature of the LAD was released. The duration of the arrhythmias was expressed as an arrhythmia severity index (ASI), being a 30-min arrhythmia considered as irreversible.[25] At the end of the protocol, the wet weight of the hearts was recorded, normalized by the tibia length and then expressed as mass index (mg/mm).

2.7. Cardiomyocyte isolation and calcium recording

Adult ventricular myocytes obtained from three Wistar rats and three WAR were isolated and stored in Dulbecco's modified Eagle medium (DMEM, Sigma–Aldrich, Inc.) until they were used (within 6 h), as previously described.[22] Incubation with 6 μM Fluo-4AM (Invitrogen, Eugene, OR) at room temperature for 30 min was performed and then the cells were washed with an extracellular solution that contained 1.8 mM Ca^{+2} to remove excess dye. Calcium transients were elicited by field stimulation of myocytes through a pair of platinum electrodes with 0.2 ms supra-threshold voltage square pulses. Cells were stimulated at 1 Hz to produce steady-state conditions. The confocal line-scan imaging was performed by a Zeiss LSM 510 META microscope (CEMEL, Institute of Biological Sciences) and a 63× oil immersion objective was used for confocal fluorescence imaging. Fluo-4 was excited at 488 nm (argon laser) and emission intensity was measured at >510 nm. For recording Ca^{2+} transients, myocytes were scanned with a 512 line. The scan line was positioned randomly along the longitudinal axis of the cell, although care was taken to avoid crossing the nuclei. Cells were scanned every 1.54 ms and sequential scans were stacked to create two-dimensional images with time on the *x*-axis. Digital image processing was performed by using custom-devised routines with IDL programming language (Research Systems, Boulder, CO) for data analysis. The calcium level was reported as F/F_0, where F is the resting calcium fluorescence inside the cell. Time to peak of the contraction and time to half-relaxation were calculated.

2.8. β-Adrenergic blockade

WAR were treated for 2 weeks with atenolol (a selective β1-adrenergic antagonist, 50 mg/kg/day i.p) or vehicle (Saline) prior to the experiments. Cardiac function, cardiac hypertrophy, reperfusion arrhythmias, and susceptibility to audiogenic seizure were evaluated in these animals.

2.9. Statistical analysis

Data are reported as mean ± SEM and the number of cells or animals by experiment is shown as *n*. Significant statistical differences were determined with Student's *t*-test. Values of $p < 0.05$ were considered to be statistically significant.

3. Results

The hemodynamic analysis showed that SAP was higher in WAR than in control rats (133 ± 3 mmHg *vs.* 121 ± 1 mmHg, $p < 0.05$, respectively). Similarly, the HR of WAR was also significantly higher than that of control rats (400 ± 6 bpm *vs.* 347 ± 11 bpm, $p < 0.05$, respectively) during the interictal period. No significant differences were observed in other hemodynamic parameters (Table 1).

The autonomic nervous system activity was analyzed through the HR responses to pharmacological blockade of muscarinic and β-adrenergic receptors by intravenously injections of methyl atropine (vagal effects) and atenolol (sympathetic effects), respectively. We found that both sympathetic activity and tone were higher in WAR when compared with control group. In addition, WAR and control rats had similar vagal tone. On the other hand, the intrinsic HR was lower in WAR (Table 2). In addition, analysis of the vagal sympathetic effect (VSE), defined as the ratio between RR interval and intrinsic RR interval during autonomic blockade, revealed that the mean VSE was 1.10 ± 0.04 in Wistar rats and 0.89 ± 0.03 in WAR ($p < 0.05$), thereby further confirming the sympathetic predominance on the sinus node in WAR.

Importantly, autonomic dysfunction observed in WAR was not linked to cardiac fibrosis (data not show). However, we found that hearts from WAR presented significant hypertrophy as observed by the analysis of the heart weight/tibia length ratio (cardiac mass index) (0.319 ± 0.006 g/cm in control rats, *n* = 17 *vs.* 0.365 ± 0.005 g/cm in WAR, *n* = 17, $p < 0.05$). We also measured the body and heart weights of 10 or 11-week old WAR and found that these animals are smaller that control rats (WAR: 266 ± 8 g and control: 390 ± 7 g). However, they presented a higher heart weight (1.35 ± 0.02 g *vs.* 1.05 ± 0.02 g, in WAR and control rats, respectively).

Table 1
Basal hemodynamic parameters in Wistar rats and Wistar audiogenic rats.

	Wistar	WAR
SAP (mmHg)	121 ± 1	133 ± 3*
DAP (mmHg)	80 ± 2	84 ± 4
MAP (mmHg)	99 ± 2	107 ± 4
PAP (mmHg)	40 ± 3	47 ± 3
HR (bpm)	347 ± 11	400 ± 6*

Values are mean ± SEM, *n* = 5 for each group. SAP, systolic arterial pressure; DAP, diastolic arterial pressure; MAP, mean arterial pressure; PAP, pulse arterial pressure; HR, heart rate.
* $p < 0.05$ compared with Wistar rats (unpaired Student's *t* test).

Table 2
Heart rate (bpm) responses to methyl atropine and atenolol injections in Wistar rats and Wistar audiogenic rats.

	Wistar	WAR
Vagal effect	100 ± 7	88 ± 23
Sympathetic effect	−31 ± 4	−111 ± 8*
Vagal tonus	58 ± 8	72 ± 10
Sympathetic tonus	66 ± 9	119 ± 15*
Intrinsic heart rate	379 ± 9	357 ± 11*

Values are mean ± SEM, *n* = 5 for each group.
* $p < 0.05$ compared with Wistar rats (unpaired Student's *t* test).

Table 3
Electrocardiographic parameters of Wistar rats and Wistar audiogenic rats.

	Wistar	WAR
RR (ms)	192 ± 4	163 ± 2*
PR (ms)	46.6 ± 0.5	47 ± 0.5
QRS (ms)	39.3 ± 0.4	43 ± 0.4*
QT (ms)	73.5 ± 0.8	85.7 ± 0.8*
QTc	173 ± 1.15	205.7 ± 1.8*

Values are mean ± SEM, *n* = 7 for each group.
* $p < 0.05$ compared with Wistar rats (unpaired Student's *t* test).

In vivo, the ECG analysis revealed that WAR presented a shorter RR interval, reflecting the higher HR seen in these animals. On the other hand, the QT interval, QTc and QRS complex were prolonged in WAR when compared with control rats (Table 3). The PR interval did not differ between the groups, indicating that the atrioventricular nodal conduction was normal in WAR (Table 3). Wistar rats presented shorter RR interval (ranging from 178 to 205 ms) compared to WAR (ranging from 158 to 172 ms). In control rats, the QRS complex ranged from 38 to 41 ms and in WAR the interval occurred between 42 and 45 ms. The QT interval of WAR was significantly longer (83–90 ms) than in control animals (70–76 ms). In WAR, despite the increased HR, QTc interval ranged from 198 to 211 ms while in control group it ranged between 168 and 177 ms. In this group, despite the increased in HR, QTc interval ranged from 198 ms to 211 ms and in control group ranged between 168 ms and 177 ms. Accordingly, *ex vivo* analysis demonstrated that the QT interval was prolonged in isolated hearts of WAR when compared with control rats (67 ± 0.8 ms in control hearts, *n* = 7 *vs.* 76 ± 0.9 ms in WAR hearts, *n* = 7, $p < 0.05$).

Fig. 2 shows a representative raw tracing of arterial pressure (mmHg) and electrical activity (mV) of isolated hearts from WAR and Wistar rats. Isolated heart preparation showed that systolic tension and HR of audiogenic rats were lower than control rats during the basal period (Fig. 3A and B, respectively). No significant changes in coronary flow were observed (6.38 ± 0.34 mL/g/min in Wistar rats *vs.* 7.25 ± 0.60 mL/g/min in WAR). Myocardial ischemia procedure induced a similar reduction in the coronary flow in all groups (approximately 50%), which was sustained throughout the ischemic period (data not shown). During the occlusion period, cardiac arrhythmias were observed in all groups. Nevertheless, the arrhythmias occurred more frequently in WAR than in control rats, as well as its duration was greater in the first group (110 ± 13 s in control rats *vs.* 189 ± 17 s in WAR, $p < 0.05$). Similarly, during the reperfusion, cardiac arrhythmias were observed in all groups. However, WAR presented irreversible arrhythmias (reperfusion arrhythmias with duration of more than 30 min), while in control hearts VT and/or VF were reversed to normal sinus rhythm in 13.87 ± 4.43 min.

In order to examine the relationship between cardiac arrhythmias and the number epileptic seizures, we evaluated the duration of arrhythmias in parallel with the augmentation of the seizure repetition. Five animals from the WAR colony without seizures induced by high-intensity sound stimulation (not submitted to a screening procedure) and five animals submitted to six audiogenic seizures were evaluated. We did not observe any significant alteration in terms of cardiac arrhythmias when the number of seizure was increased (data not shown). These data demonstrated that WAR rats have an inherently greater susceptibility to arrhythmias.

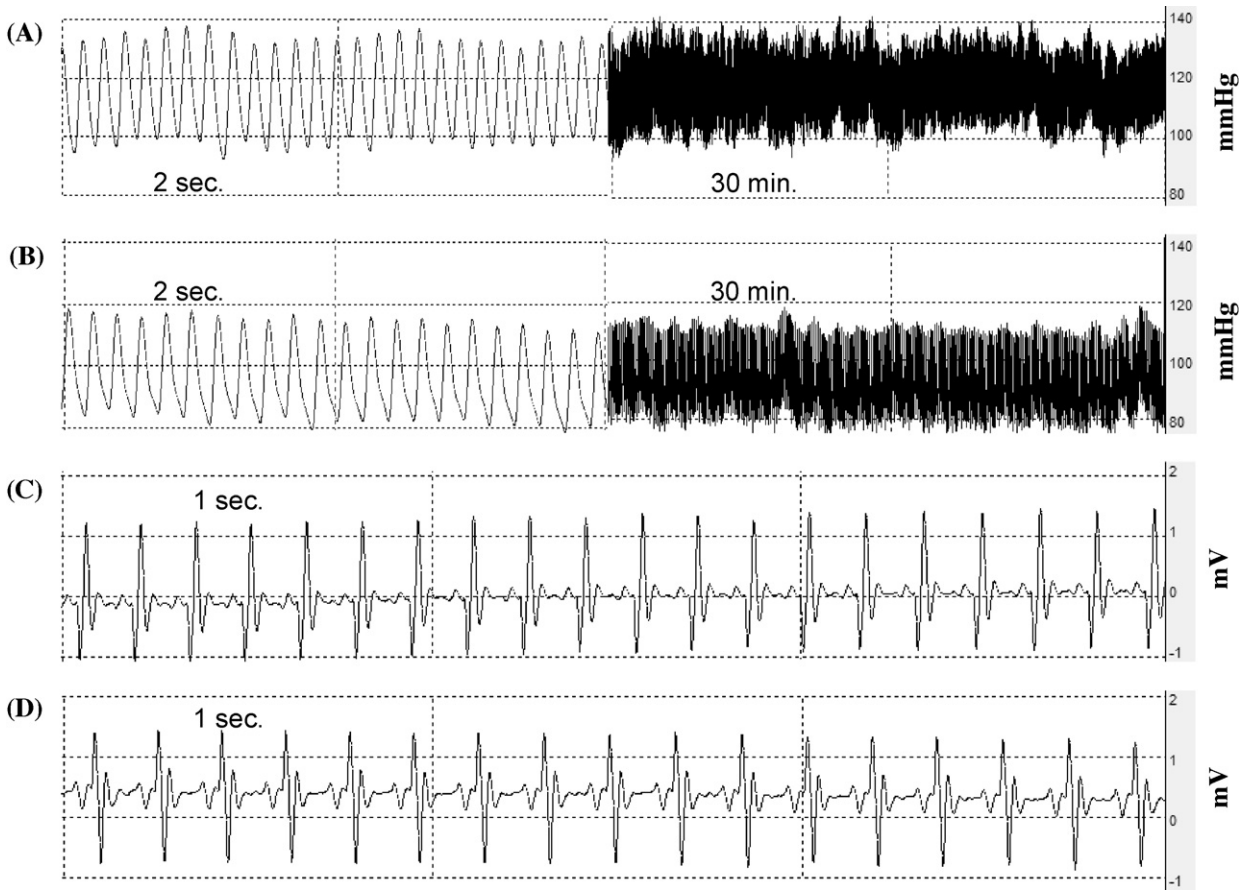

Fig. 2. Representative raw tracings of arterial pressure (mmHg) and of electrical activity (mV) of isolated hearts of WAR (A and C) and of Wistar rats (B and D).

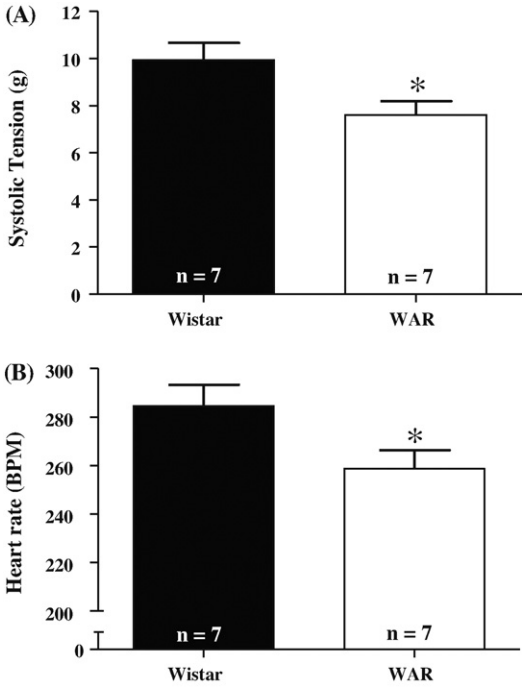

Fig. 3. Cardiac function of Wistar rats and WAR evaluated by an isolated heart preparation. (A) Systolic tension and (B) Heart rate. Values are mean ± SEM. *$p < 0.05$ compared with Wistar rats (Student's t test). n indicates the number of animals analyzed.

We also investigated the atenolol effects on cardiac hypertrophy, cardiac function and arrhythmias of reperfusion. The hypertrophy observed in WAR hearts (0.359 ± 0.008 g/cm, $n = 7$) was reversed by the treatment with atenolol (0.256 ± 0.007 g/cm, $n = 7$, $p < 0.05$). In terms of heart function, isolated hearts of audiogenic rats showed lower systolic tension when compared with control rats during the basal period. In addition, contractile function of WAR hearts was improved by atenolol (7.61 ± 0.26 g in WAR *vs.* 10.01 ± 0.58 g in WAR + atenolol, $n = 7$, $p < 0.05$). No significant changes in HR and coronary flow were observed after the treatment. In addition, the reperfusion arrhythmias persisted for more than 30 min after the ligature release in WAR hearts, characterizing an irreversible arrhythmia. In the other hand, hearts from WAR + atenolol VT and/or VF returned to normal sinus rhythm in 21.3 ± 3.9 min ($p < 0.05$, $n = 7$). No significant changes in susceptibility to audiogenic seizure were observed between the groups.

Altered Ca^{2+} handling can influence cardiomyocyte physiology and changes in intracellular Ca^{2+} are a major feature in myocyte pathology. Therefore, to determine if the heart dysfunction detected in WAR was related to changes in Ca^{2+} handling, we examined intracellular Ca^{2+} in freshly isolated Fluo-4-loaded ventricular myocytes from Wistar rats and WAR. Fig. 4A shows that myocytes from WAR present a significant elevation of the Ca^{2+} transient peak (approximately 16%, $p < 0.05$, $n = 67$ cells from three different animals in each group). These changes were associated with alterations in the kinetics of the Ca^{2+} decay (time to 50% decay and time to 90% decay, Fig. 4B and C, respectively).

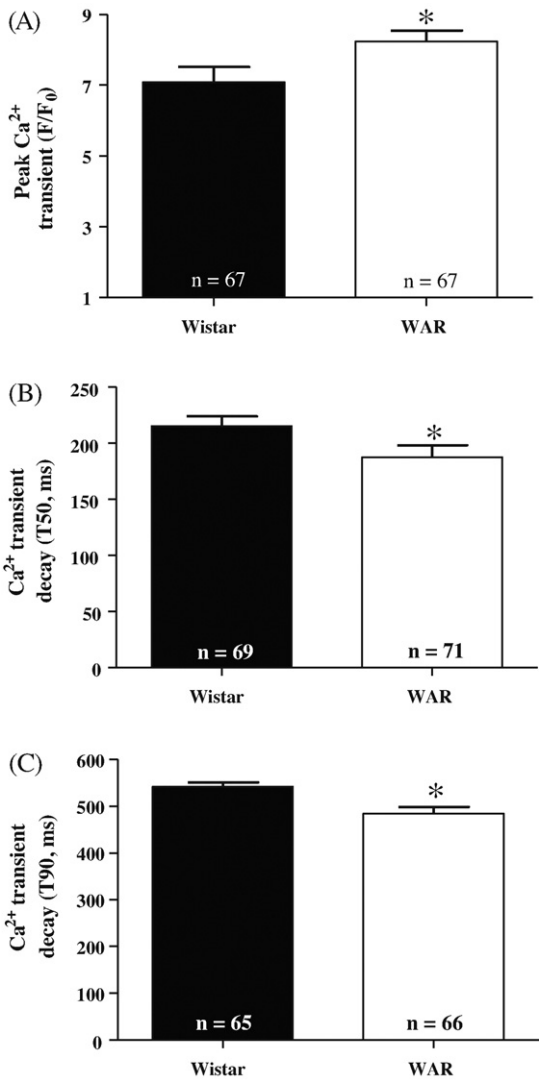

Fig. 4. Calcium transient parameters of ventricular cardiomyocytes of Wistar rats and WAR. (A) Calcium transient amplitude peak and (B and C) calcium transient decay kinetics. Data are shown as the ratio of measured fluorescence (F) over basal fluorescence (Fo). Values are mean ± SEM. $^{*}p < 0.05$ compared with Wistar rats (Student's *t* test). T50 and T90 represent the time from peak Ca^{2+} transient to 50% and 90% decay, respectively. *n* indicates the number of cardiomyocytes analyzed.

4. Discussion

Lara et al.[26] provided direct evidence that decreased cholinergic neurotransmission and autonomic imbalance cause plastic alterations that might contribute to heart dysfunction. Therefore, based on this we investigated the consequences of increased sympathetic tone in the cardiovascular function of rats prone to audiogenic seizure (WAR) using *in vivo* (hemodynamic, autonomic tone and ECG), *ex vivo* (isolated perfused hearts – cardiac function and incidence of ischemic/reperfusion arrhythmias) and *in vitro* (intracellular calcium handling) approaches. Overall, we presented evidence that chronic disturbances in autonomic tone in WAR induced cardiac structural alterations with changes in SAP, ventricular function and increases in basal HR, intracellular Ca^{2+} handling and arrhythmias following ischemia/reperfusion injury.

Left ventricular hypertrophy is associated with a higher incidence of death and is a harbinger of morbidity and mortality by cardiovascular disease.[27] Thus, the susceptibility of WAR to develop cardiac arrhythmias could be a consequence of the cardiac hypertrophy observed in these rats since hypertrophied hearts display a greater incidence of sustained VF.[28] Furthermore, the increased basal SAP, HR and sympathetic tone observed in WAR might contribute, at least in part, to the development of cardiac hypertrophy.

Abnormal cardiac autonomic control including high levels of sympathetic activity and impaired parasympathetic control is associated with cardiac dysfunction[26,29] and predisposes the heart to VF.[30] It has been demonstrated that vagal activation increases the threshold to trigger VF induced by sympathetic stimulation.[31] Moreover, Ng et al.[32] have found that sympathetic stimulation decreases the effective refractory period, as well as the VF threshold. Thus, the higher sympathetic tone viewed in WAR can probably induce structural alterations in the heart that may contribute to the increase in cardiac arrhythmias following ischemia/reperfusion injury observed in isolated hearts of these animals.

It was observed, both *in vivo* and *in vitro*, that WAR presented a prolonged QT interval when compared with control rats. The QT interval reflects depolarization and repolarization of myocardial cells. Factors that augment depolarization or delay repolarization of myocardial cells can increase QT interval length. In accordance to Bruyne et al.[33], prolonged QTc and QT are both predictors of an increased risk of ventricular arrhythmias and sudden cardiac death in humans. In addition, increases in QTc values during EEG epileptiform discharges were observed by Tavernor et al.[34] in a group of patients who subsequently died of sudden unexplained death.

Ex vivo analysis demonstrated that the QT interval was prolonged in isolated hearts of WAR when compared with control rats (67 ± 0.8 ms in control hearts, $n = 7$ *vs.* 76 ± 0.9 ms in WAR hearts, $n = 7$, $p < 0.05$). Furthermore, our data showed abnormal intracellular Ca^{2+} transient of WAR cardiomyocytes (Fig. 4) and altered electrical activity (prolonged QT interval) during *in vivo* ECG measurements (surface ECG). Thus, the elevation in the amplitude of the intracellular calcium transient could be a result of cardiac action potential (AP) prolongation.[35] In fact, this prolongation in ventricular cell is a major determinant of the QT interval and of factors that alter the balance between inward and outward currents during the phase 2 of the AP.[36]

It has been suggested that an imbalance in the sympathetic nervous system may result in prolongation of the QT interval in ECG tracings.[30] In fact, patients with prolongation of the QT interval have a predisposition for the development of life threatening ventricular arrhythmias.[37] The lethal arrhythmias associated with prolongation of the QT interval are almost always *torsade-de-pointes* ventricular tachycardia due to early after depolarization and are usually triggered by sudden increases in sympathetic activity.[30]

The long QT syndrome (LQTS) is characterized by the appearance of long QT intervals in the ECG and a relatively high risk for sudden cardiac death.[38] QT prolongation during isoproterenol treatment has been reported in patients with LQTS.[39] In accordance to Zipes,[38] LQTS may be the Rosetta Stone for ventricular tachyarrhythmias dependent on sympathetic stimulation and Antzelevitch[40] has provided important insights into different sensitivities of different genotypes of long QT syndrome to sympathetic stimulation. In addition, mutations in potassium-channel genes were the first genetic causes of LQTS to be identified.

Mesquita et al.[41] observed that the amplitudes of the outward stationary K^+ currents and transient K^+ current in hippocampal neurons are significantly smaller (about 30%) in WAR than in controls. The resting membrane potential in WAR is significantly more depolarized (−50 mV) than that of the controls (−63 mV) and input resistance is altered in these animals (647 MΩ in WAR and 408 MΩ in controls). In addition, functional knockout of I_{to} (transient outward K^+ current) leads to marked increases in AP

durations in ventricular myocytes and to prolongation of the QT interval recorded during surface ECG.[42]

Furthermore, WAR also presented an increase in the duration of the QRS complex, which could be attributed to the higher cardiac muscle mass observed in these animals.[43] In keeping with these findings, it has been reported that the left ventricular hypertrophy observed in spontaneously hypertensive rats (SHR) is accompanied by an increase in the QRS duration.[44]

Isolated heart preparation also demonstrated that audiogenic rats present a lower systolic tension, despite the increased peak Ca^{2+} transient observed in isolated ventricular cells of WAR. Similar results have been reported, for example, the active tension of papillary muscle was significantly depressed and the peak of Ca^{2+} was increased in SHR with cardiac hypertrophy.[45,46]This study provided evidence that WAR presented an augmented tonic sympathetic activity *in vivo*. β-adrenergic activation of the heart is a pro-arrhythmogenic event known to increase sarcoplasmic reticulum Ca^{2+} load and the frequency of spontaneous sarcoplasmic reticulum Ca^{2+} release.[47] The increase in $[Ca^{2+}]i$ transients in response to β-adrenoceptor stimulation is in part mediated by an increase in L-type Ca^{2+} current, leading to a greater Ca^{2+} influx and an increased release of Ca^{2+} from the sarcoplasmic reticulum during the AP.[48] Defective cellular calcium handling, due to abnormalities of the various components, which mediate and control excitation-contraction coupling, is widely recognized as a significant mediator of certain forms of cardiac arrhythmias.[49] Myocyte relaxation is a result of the rapid removal of the cytosolic Ca^{2+} by its re-uptake into the sarcoplasmic reticulum through the sarcoplasmic reticulum Ca^{2+}/ATPase pump (SERCA), among other factors.[50] In the present study, it was observed an increase in the peak amplitude of the $[Ca^{2+}]_i$ transients in WAR. On the other hand, decay to 50% amplitude (RD50), which estimates the rate of decay of the $[Ca^{2+}]_i$ transient following a contraction, was also altered, indicating a faster re-uptake in this group of cells.

WAR cardiomyocyte cells had a significant elevation in the magnitude of the Ca^{2+} transient and this ion may play a primary role in the induction of arrhythmias upon reperfusion.[51] Alterations in Ca^{2+} influx probably are also involved in the initiation of VT/VF after reperfusion in WAR. Indeed, Brooks et al.[52] showed that VT/VF after myocardial reperfusion is immediately preceded by a large increase in the amplitude of the Ca^{2+} transient, thereby indicating that alterations in the intracellular Ca^{2+} regulation possess a significant role in initiating these arrhythmias.

Abnormal Ca^{2+} handling has been implicated in arrhythmogenesis in numerous pathologies and the role of spontaneous systolic Ca^{2+} release from sarcoplasmic reticulum (SR) in the generation of early after depolarizations (EADs) continues to be debated.[53] Nemec et al.,[54] in this study indicate that secondary $[Ca^{2+}]_i$ transient peaks are caused by instabilities of intracellular Ca^{2+} handling caused by AP prolongation. These instabilities in turn promote the appearance of EADs, and eventually torsades de pointes. Ventricular tachycardias may follow the AP prolongation associated with reduced K^+ channel expression that, in turn increases the likelihood of late L-type Ca^{2+} channel ($I_{Ca,L}$) reactivation. Furthermore, increased sensitivity of Na^+/Ca^{2+} exchange to any spontaneous release of intracellularly stored Ca^{2+} from the sarcoplasmic reticulum (SR) increases transient, and potentially arrhythmogenic, inwards currents (I_{ti}).[55] Reperfusion arrhythmias thus represent another clinically relevant situation involving spontaneous Ca^{2+} release from overloaded SR.[54]

Treatment with atenolol (β1-adrenergic antagonist) increased the systolic tension and prevented cardiac hypertrophy, as well as the associated increase in susceptibility to experimentally induced arrhythmias in WAR. These results suggest that the blockade of cardiac adrenoreceptors during interictal period would protect the heart from events that increase the risk to arrhythmias and improves the cardiac function. In the present study, atenolol showed no effects on susceptibility to audiogenic seizures. Similarly, De Sarro et al.[56] observed that atenolol did not alter seizure activity or effectiveness of anticonvulsant agents.

In summary, our present data showed that WAR rats present an increased basal HR and SAP associated with changes in the sympathetic tone, which may favor the development of cardiac hypertrophy. This phenotype might contribute to the alterations in the ECG observed in these animals. Prolongation of the QT interval associated with elevation in the peak amplitude of Ca^{2+} may increase the risk of life-threatening ventricular arrhythmias. Therefore, WAR holds a strong tendency to develop cardiac arrhythmias and present an increase in the arrhythmias following ischemia/reperfusion injury. Altogether, these results support a relationship among seizures, cardiac dysfunction and cardiac arrhythmias. This relationship may partially account for the occurrence of SUDEP.

Conflict of interest statement

None of the authors has any conflict of interest to disclose.

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